Carcinogenesis in Swiss Mice by Isonicotinic Acid Hydrazide

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Summary

Administration of 0.1% isonicotinic acid hydrazide (INH) in drinking water to 9-week-old Swiss mice for the remainder of their lifetime resulted in the enhancement of pulmonary adenomas. The incidence rose from 14 to 53% in the females and from 10 to 42% in the males, as compared with the controls. The incidence of other tumors in these animals was not increased.

Introduction

There have been several demonstrations of the limited tumorigenicity of INH in mice. The 1st such experiment was reported by Juhász et al. (3, 4), who reported the induction of pulmonary adenomas, lymphomas, and leukemias in randomly bred mice injected i.p. with INH. In a subsequent study these same investigators found only an induction in lymphomas and leukemias but reported the occurrence of only 1 pulmonary adenoma (5). Other workers have reported on the induction of pulmonary adenomas (1, 7, 8, 10, 11) and it has more recently been suggested that this action may be mediated through hypothetical metabolism of INH to hydrazine sulfate, which has also been shown to induce lung adenomas (2) in BALB/c mice. In 1 study in the rat (9) no tumors were induced with repeated injections of INH and, in fact, in another study in this species the production of hepatomas by p-dimethylaminoazobenzene was inhibited by combined administration of this carcinogen with INH (6). The clinical implications of these various findings have been discussed and many divergent viewpoints presented.

The present study has been undertaken because of the clinical interest in this situation. Prior studies reported were terminated before the end of the life-span of the mice. It was felt that additional information might be obtained if the experiments were allowed to run to their conclusion.

Materials and Methods

Swiss albino mice were used from a colony originally obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and bred randomly in this laboratory since 1951. They were housed in plastic cages with sterilized granular cellulose bedding, separated according to sex in groups of 10, and were given Rockland diet in pellets and tap water ad libitum with the exception to be described.

The drug used was isonicotinic acid hydrazide (isoniazid, INH, Eastman Organic Chemicals, Rochester, New York). The experimental and control groups and the treatment are described as follows.

GROUP 1. INH was dissolved in the drinking water as a 0.1% solution and was given continuously for the life-span of 55 female and 55 male Swiss mice which were 9 weeks old at the beginning of the experiment. This dose level was selected because other investigators have employed it as an effective carcinogenic treatment, and because we obtained a rapid toxic effect with a 0.2% solution of INH. The solution was prepared twice a week and the total water consumption with INH in it was measured at the same intervals during the treatment period. The average daily water consumption with INH in it per animal was 4.7 ml for the females and 5.5 ml for the males. The average daily intake of INH, therefore, was 4.7 mg for the females and 5.5 mg for the males; the INH consumption during the whole course of the experiment (terminated at the time the last animal died) was approximately 2.646 gm/female and 3.096 gm/male.

GROUP 2. As a control, 110 females and 110 male mice were kept untreated.

The experimental and control animals were carefully checked and weighed at weekly intervals, and the skin and subcutaneous changes recorded on graph paper. The animals were allowed to die spontaneously or were killed with ether when found in poor condition. Complete necropsies were performed on all animals except those lost through cannibalism. All organs were examined macroscopically and were fixed in 10% buffered formalin. Histologic studies were done on the liver, spleen, kidneys, and at least 4 lobes of the lungs of each mouse, as well as on those organs which showed gross pathologic changes. Sections from these tissues were stained routinely with hematoxylin and eosin and with additional special methods when necessary.

In tabulating our results, we determined the latent periods of malignant lymphomas and visceral tumors from the birth of the animal to the time of death. The latent periods of skin and subcutaneous tumors were based on the time at which they were 1st recognized grossly in the live animal. Percentage figures in the tables were based on the original number of animals at the start of the experiment.

Results

The average weight curves of the treated and control animals are shown on a weekly basis (Chart 1). It is evident that the treatment with INH resulted in a reduction of body weight. The survival rate at 10-week intervals is recorded in Table 1, which shows that administration of INH significantly reduced survival rate.
The number, incidence, and latent period of the tumors are summarized in Table 1. In the INH-treated group, 23 females developed lung tumors with an incidence of 53.4%, excluding 12 mice of which 11 were partially and 1 completely eaten since in the 1st instance their chest organs with the lungs were missing. The average latent period for these lung tumors was 70.3 weeks, the 1st appearing at the 24th week and the last at the 89th week. For the 21 lung adenoma-bearing, treated males the incidence was 42.0%, excluding 5 partially eaten mice. Their average latent period was 65.6 weeks, the 1st lung tumor appearing at the 45th week and the last at the 89th week.

In the control group, 14 females developed lung tumors. The incidence was 14.2%, excluding 12 partially eaten mice. The average latent period was 90.5 weeks, the 1st appearing at the 64th week and the last one at the 119th week. Among the control males, 11 mice developed lung tumors with an average latent period of 74.2 weeks, an incidence of 10.1%. The 1st tumor appearing at the 47th and the last at the 110th week, excluding 2 partially eaten animals.

A microscopic diagnosis was made of every lung tumor, and the results with time of appearance in both the treated and control groups are presented in Chart 2.

There were certain numbers of other tumors in the experimental and control groups which in accordance with our earlier observation concerned spontaneous tumor types in this strain of mice (13). It should be noted, moreover, that in 5 treated females subcutaneous nodules appeared on the abdominal region or on either side of the body at 21, 28, 49, 49, and 49 weeks, and all regressed several weeks later. They were probably breast tumors. In the control females 9 developed breast neoplasms, of which 9 were classified as adenocarcinomas and appeared at 46, 60, 64, 68, 73, 91, 93, 98, and 110 weeks, respectively. In addition, in this group 3 mice developed subcutaneous fibrosarcomas at 68, 82, and 82 weeks and 1 had a subcutaneous fibroma at the 87th

### Table 1

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TREATMENT</th>
<th>INITIAL NO. AND SEX OF ANIMALS</th>
<th>NO. OF SURVIVORS AT WEEKS OF AGE</th>
<th>LUNG TUMORS</th>
<th>MALIGNANT LYMPHOMAS</th>
<th>MAMMARY TUMORS</th>
<th>OTHER TUMORS</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 20 30 40 50 60 70 80 90 100 110</td>
<td>No.</td>
<td>%</td>
<td>Latent period (wk.)</td>
<td>No.</td>
</tr>
<tr>
<td>1</td>
<td>0.1% INH in drinking water for life-span</td>
<td>55 ♀</td>
<td>54 47 46 41 39 32 14 5 0 0 0</td>
<td>23</td>
<td>83.4</td>
<td>70.3 (34-89)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>55 ♂</td>
<td>55 49 48 44 37 15 10 3 0 0 0</td>
<td>21</td>
<td>42.0</td>
<td>45.6 (45-89)</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Untreated control</td>
<td>110 ♀</td>
<td>100 106 107 104 96 89 73 57 41 23 11 1</td>
<td>14</td>
<td>14.2</td>
<td>90.5 (64-110)</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>110 ♂</td>
<td>93 91 86 67 55 41 22 6 1 1 0</td>
<td>11</td>
<td>10.1</td>
<td>74.2 (47-110)</td>
<td>2</td>
</tr>
</tbody>
</table>

a INH, isonicotinic acid hydrazide.
b A, 1 hemangams of liver at 89th week, 1 carcinoma of vagina at 75th week. B, 1 malignant papilloma at 78th week, 1 luteoma at 99th week, 1 granulosa cell tumor at 60th week, 1 sex cord mesenchyma tumor at 99th week, 1 hemangias of ovary at 42nd week, 3 hemangias of livers at 89th, 77th, and 84th weeks, 3 subcutaneous sarcomas at 60th, 52nd, and 42nd weeks, 1 subcutaneous fibroma at 87th week, 1 papilloma of forestomach at 112th week. C, 2 hemangias of livers at 73rd and 80th weeks.

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**Table 1**

**Survival Rate and Distribution Pattern of Tumors in Isoniazid-treated and Control Swiss Mice**

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**Chart 2.** The appearance of lung tumors in the isoniazid treated and control mice. Each symbol represents an animal with: lung adenomas (●); lung adenocarcinomas (★); lung adenomas and adenocarcinomas (▲); pulmonary adenomatosis (○).
Isonicotinic Acid Hydrazide Administration in Mice

week. Since in these 4 tumors there was no histomorphologic evidence of mammary gland tissue, they were considered to be subcutaneous in origin.

The appearance of malignant lymphomas was similar to that previously reported by us (14) and these were further classified as follows: In the treated females and males, 2 were of the lymphocytic type. In the control females, there were 5 lymphocytic types at 39, 45, 48, 69, and 77 weeks, 1 mixed cell type at the 72nd week, 8 histiocytic types at 72, 84, 85, 89, 96, 98, 102, and 115 weeks, and 2 unclassifiable at 56 and 98 weeks. Among the control males there was 1 lymphocytic type at the 73rd week and another was unclassifiable because of advance decomposition at the 82nd week.

Discussion

The present study has confirmed previous reports that large amounts of isonicotinic acid hydrazide will result in an increased number of lung adenomas in mice. Contrary to the findings of some other workers (4, 5), there was no increase in the number of lymphomas and leukemias compared with the controls. In fact, our results suggest that the number of mammary tumors and possibly the lymphomas might even be decreased in the treated group as compared with the controls; this possibility is made even more likely by the fact that several probable mammary tumors appeared during the course of the study in the treated animals only to regress.

The finding that an agent will enhance the incidence of 1 commonly occurring tumor and at the same time have no carcinogenic effect on 2 other types, or rather have an inhibitory action on some tumor growth, is in our experience unusual. The dose of INH employed was toxic, and in this study there was somewhat of a weight decrease in the treated animals. This, from the many studies of Tannenbaum and Silverstone (12) might account for the decrease or lack of enhancement of mammary tumors and lymphomas, but it is difficult to account, at the same time, for the increased incidence of lung adenomas. Once again this study emphasizes the individuality of single types of tumor both in the factors that may enhance their occurrence and in the factors that may inhibit their progression and growth.

INH appears to be a weak and limited carcinogen from the findings so far available. It is active only in the mouse and then apparently for only 1 tumor type. Before any conclusions on the clinical implications of such studies are drawn it would be prudent to test this agent under many other conditions in different species and ensure that clinical material available be utilized to the fullest extent.

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References

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